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TITLE: The Role of aVb6-mediated latent TGF-B1 Activation in  
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<b>13. ABSTRACT (Maximum 200 Words)</b> Abundant evidence suggests that overexpression of TGF- $\beta$ by prostate cancer cells enhances their ability to grow and metastasize. TGF- $\beta$ is secreted by cells in a latent form that results from a noncovalent interaction between TGF- $\beta$ and its propeptide (latency-associated peptide, LAP). Mechanisms leading to active TGF- $\beta$ are poorly understood at present. Our lab discovered a mechanism of TGF- $\beta$ activation in which the integrin $\alpha$ V $\beta$ 6 binds to an RGD sequence near the C-terminus of LAP. $\alpha$ V $\beta$ 6 is only expressed in epithelial cells. We hypothesize that $\alpha$ V $\beta$ 6, by activating TGF- $\beta$ 1, is an important regulator of normal prostate epithelial proliferation, and that overexpression of $\alpha$ V $\beta$ 6 by prostate tumor cells acts in concert with overexpression of its ligand latent TGF- $\beta$ 1 to produce active TGF- $\beta$ 1 and promote growth of the tumor. In this work, we are testing whether the $\beta$ 6 integrin subunit is regulated by androgen, whether it is overexpressed in human prostate cancer, and whether it affects growth and metastasis of prostate cancer in an animal model. Our results to date indicate that $\beta$ 6 expression is upregulated in the mouse in a delayed fashion after castration. We are now testing whether castration-induced prostate involution is affected by $\beta$ 6 by comparing normal and $\beta$ 6 KO animals, and producing mice that develop prostate cancer (TRAMP) that also lack $\beta$ 6 gene expression.				
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## INTRODUCTION

The subject of this work is a system for the activation of latent TGF $\beta$  in the prostate. The system consists of the  $\alpha$ V $\beta$ 6 integrin expressed on epithelial cells. Our previous work showed that this integrin can bind to an integrin recognition site (arg-gly-asp) on latent TGF $\beta$ 1 and effect its activation [1]. TGF $\beta$  is known to be important for regulating the growth and differentiation of various epithelia, and also to be important in cancer growth. Little is currently known about this system in the prostate: eg, what cells express  $\alpha$ V $\beta$ 6, how expression of the integrin is regulated, and if and when this system regulates prostate epithelial growth via production of active TGF $\beta$ 1. The purpose of the work is to demonstrate whether or not this system plays a role in prostate cancer. The scope of the work involves cell line and mouse experiments (to gauge the normal expression and regulation of  $\alpha$ V $\beta$ 6 in the prostate), and evaluation of human prostate cancer tissue and an *in vivo* mouse prostate cancer model (to address the question, does  $\alpha$ V $\beta$ 6-mediated activation of TGF $\beta$ 1 promote prostate cancer growth?).

## BODY

The second 12 months of the project addressed tasks 1 (determine  $\beta 6$  expression in normal prostate and prostate cancer cells), 2 (determine the effect of androgen on  $\beta 6$  expression), 3 (effect of  $\beta 6$  on prostate epithelial proliferation) and 5 (effect of  $\beta 6$  on cancer in a mouse model of prostate cancer).

We have castrated control and  $\beta 6$  KO mice and sacrificed them at multiple time points. We have also BrDU-labeled control and KO mice. We now have sufficient samples to analyze and are systematically analyzing the samples. We are doing TUNEL staining of the involuting prostate specimens to see if  $\beta 6$  expression alters the rate of apoptosis. Preliminary results suggest that at the day 3 time point there is less apoptosis in the KO mice. The only remaining samples to generate are exvoluting prostates in normal and KO mice (castrated mice are given DHT pellets to induce regrowth of the prostate).

We have obtained TRAMP mice and are currently crossing them with the  $\beta 6$  KO mice. Once we generate combined  $\beta 6$ KO/TRAMP (estimate: 2-3 months) mice we will assess their tumor growth and metastasis.

We have looked at  $\beta 6$  expression in human prostate cancer tissue. In normal areas,  $\beta 6$  is expressed in basal cells. In the cancer areas, we see no  $\beta 6$  expression except in occasional intensely stained cells. The tumors are of intermediate grades and we are trying to get samples of higher grade tumors as well as of metastases to see if  $\beta 6$  expression is a marker of more aggressive cancer.

## KEY RESEARCH ACCOMPLISHMENTS

- $\beta 6$  is expressed in basal cells of human prostate. This may be important for growth control of these cells (via  $TGf\beta$  activation) and might be involved in growth control of prostate stem cells, which may exist in this compartment.
- Human prostate cancer, at least of intermediate grade, does not express  $\beta 6$  except for rare positive cells. This may be because these lower grade tumors are still growth-inhibited by  $TGF\beta$ , or because they are of luminal origin.

## REPORTABLE OUTCOMES

The human prostate data are reportable.

## CONCLUSIONS

These experiments are the first to describe  $\beta 6$  expression in human prostate cancer, and in normal prostate. The remaining work done has not yet been fully analyzed but should give insights into the role of  $\beta 6$  integrin on prostate epithelial cell proliferation during androgen withdrawal and replacement.

"So what." There are many reports in the literature relating cancer outcomes and tumor cell behavior to increased expression of TGF $\beta$  by tumor cells. However, to my knowledge there has never been an analysis of the role of a TGF $\beta$  activator in tumor cells. Yet, our results with  $\alpha V\beta 6$  and lung fibrosis [1] point out the critical role that a TGF $\beta$  activator can play in a TGF $\beta$ -dependent process. If a specific TGF $\beta$  activator can be identified as important in a cancer, this knowledge might be important for determining prognosis and for developing therapies in which the activator is a target.



## REFERENCES

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